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Award Number: DAMD17-98-1-8062

TITLE: Interindividual Differences in Metabolism of Carcinogens as a Risk Factor for Breast Cancer

PRINCIPAL INVESTIGATOR: Regine Goth-Goldstein, Ph.D.

CONTRACTING ORGANIZATION: University of California at Berkeley
Berkeley, California 94720

REPORT DATE: September 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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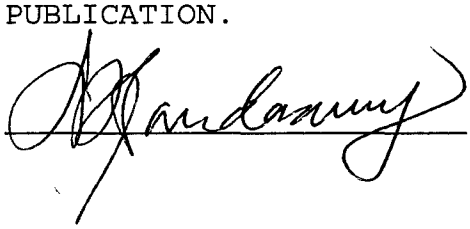

04/22/01

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2000	Annual (17 Aug 99 - 16 Aug 00)

4. TITLE AND SUBTITLE
Interindividual Differences in Metabolism of Carcinogens as Risk Factor for Breast Cancer

5. FUNDING NUMBERS
DAMD17-98-1-8062

6. AUTHOR(S)
Regine Goth-Goldstein, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of California at Berkeley
Berkeley, California 94720

E-MAIL:
r_goth-goldstein@lbl.gov

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
This report contains colored photos

12a. DISTRIBUTION / AVAILABILITY STATEMENT
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13. ABSTRACT ***(Maximum 200 Words)***
The proposed study seeks to address the interaction of environmental and genetic factors in the etiology of breast cancer. The cytochrome P450 isozymes CYP1A1 and CYP1B1 metabolize environmental and endogenously formed carcinogens in the breast. We are testing the hypothesis that individuals with higher levels of CYP1B1 are at a higher risk for breast cancer because they produce higher amounts of ultimate carcinogen. Genetic polymorphism and expression level of *CYP1A1* and *CYP1B1* are being determined in a collection of nontumor breast tissue samples from reduction mammoplasties and from mastectomy patients. Frequency of genetic variants and expression is compared in specimens from cancer patients and healthy controls to establish if breast cancer patients have an increased level of the enzyme. During the last year *CYP1B1* and in parallel *CYP1A1* expression was determined in an additional 29 specimens using a semiquantitative RT-PCR. All available specimens were analyzed for known genetic polymorphisms in the *CYP1A1* and *CYP1B1* genes. The difference in variant frequencies between cases and controls was not significant. CYP1B1 transcript levels ranged from 1.5 to 133. CYP1A1 level had an even larger interindividual range. In most specimen *CYP1B1* expression was 2-6 fold that of *CYP1A1*. *CYP1B1* expression was significantly higher in the breast cancer group than in the healthy control group.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
34

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Interindividual Differences in Metabolism of Carcinogens as a Risk Factor for Breast Cancer

Introduction

Numerous studies indicate that exposure to polycyclic aromatic hydrocarbons (PAHs) increases the risk of developing certain types of human cancers (1). The major pathway by which ingested or inhaled PAHs are metabolized, is the stepwise oxidative activation by the cytochrome P450 isozymes, CYP1A1 and CYP1B1, followed by detoxification by phase II enzymes (2). The highly reactive intermediates formed by CYP1A1 or CYP1B1 can bind to DNA, and the resulting DNA adducts can cause a mutation that if in a relevant gene could initiate cancer. Expression of both CYP1A1 and CYP1B1 is highly inducible by PAHs and other environmental toxins, such as dioxin (3). Whereas CYP1A1 has been studied extensively for over 25 years, much less is known about CYP1B1, one of the newest members of the P450 family. There is considerable evidence now that CYP1B1 could be a key enzyme in the activation of carcinogens in the breast and therefore play a role in the development of breast cancer. The CYP1B1 gene is highly expressed in human breast tissue, but not in liver which has been considered the major site for metabolism of xenobiotic compounds (4). Experiments with recombinant human enzymes showed that CYP1B1 is the principal enzyme in catalyzing oxidation of benzo[a]pyrene to the diolepoxide, being ten times more efficient than CYP1A1(5). When investigating 7,12-dimethylbenz(a)anthracene-induced lymphomas in mice, the frequency of lymphomas was reduced to one tenth in CYP1B1 null mice compared to wild-type mice (6) and CYP1B1 null mice were protected from the bone marrow cytotoxic effects (7), indicating that CYP1B1 is critical for carcinogenesis by certain PAHs and that extrahepatic metabolism is important in determining susceptibility to PAHs. CYP1A1 and CYP1B1 are also involved in estrogen hydroxylation, but they differ in the position of hydroxylation; whereas CYP1A1 acts at the C-2 position, CYP1B1 acts at the C-4 position leading to formation of the potentially carcinogenic 4-hydroxy estradiol (8).

We are testing the hypothesis that individuals with higher levels of CYP1B1 are at a higher risk for breast cancer because they produce higher amounts of ultimate carcinogen. The expression level of *CYP1B1* is being determined in a collection of normal breast tissue samples from reduction mammoplasties and from mastectomy patients and *CYP1B1* expression is compared in specimen from cancer patients and healthy controls to establish if breast cancer patients have an increased level of the enzyme.

Body of Annual Report

Interindividual variation in carcinogen metabolism has been recognized as an important determinant of susceptibility to various cancers. We are testing the hypothesis that the level and activity of enzymes with the capacity to activate environmental carcinogens in the breast represent a risk factor for breast cancer, and specifically that individuals with

higher levels of CYP1B1 are at a higher risk for breast cancer because they produce higher amounts of ultimate carcinogens. Interindividual variation in *CYP1B1* expression can be due to genetic polymorphism either in the structural gene or in a regulatory gene. Besides genetic background, various factors can modify expression of *CYP1A1* and *CYP1B1* in an individual, including hormonal levels, dietary and smoking habits, and exposure to other foreign compounds that act as inducers or repressors. Since expression is the result of these various factors, studies on a particular genetic polymorphism capture only a fraction of the enzyme variability in at risk individuals. We therefore decided to determine expression of *CYP1B1* in the breast to capture all possible modifying factors. A collection of histologically normal breast tissue specimens from mastectomy patients and from reduction mammoplasties was available and is being analyzed both for expression of the *CYP1A1* and *CYP1B1* genes and known polymorphisms in these genes.

Task 1. Expression of *CYP1B1* in healthy individuals and breast cancer patients

We determined expression of the *CYP1B1* gene and in parallel the *CYP1A1* gene by a semiquantitative RT-PCR assay in an additional 29 specimens of our collection of nontumor breast tissue from mastectomy patients and from reduction mammoplasties. The results (summarized in Figure 1 - 3 in the Appendix) show that (1) CYP1B1 levels relative to actin ranged from 1.5 - 133, (2) *CYP1B1* is expressed at higher level than *CYP1A1* in most samples. For most samples CYP1B1 transcript levels were 2-7 times higher than CYP1A1, and (3) more specimen with high *CYP1B1* expression are among the breast cancer patients than among healthy controls. The values were analyzed by Student's t-test. Whereas the difference in CYP1A1 values between the study groups did not achieve statistical significance ($p = 0.1798$), the differnce in CYP1B1 values between the study groups was statistically highly significant ($p = 0.0043$).

Table I Comparison of CYP1A1 and CYP1B1 expression in nontumor breast tissue of breast cancer patients and healthy controls

	Cancer patients	Controls
CYP1B1/β-actin, mean	41.84*	20.01*
CYP1B1/β-actin, median	29.72	12.23
CYP1A1/β-actin, mean	10.44**	6.35**

CYP1A1/β-actin, median	4.59	4.43

* statistically significant, in two-tailed t-test t = -3.094, p = 0.0043
**statistically not significant, in two-tailed t-test t = -1.35, p = 0.1798

The findings indicate that CYP1B1 is the primary enzyme for PAH metabolism in the breast and might therefore have a role in PAH-carcinogenesis. The results support our hypothesis, that individuals with higher levels of CYP1B1 are at a higher risk for breast

Task 2. To determine *CYP1A1* and *CYP1B1* genotype in cases and controls

***CYP1B1*:** One of the original goals of this project had been to try to identify *CYP1B1* polymorphisms from specimens that have high CYP1B1, but low CYP1A1 transcript levels. However, since initiating this project several polymorphisms in the *CYP1B1* gene have been reported (10-12). Some polymorphisms that result in frameshift and missense mutations, have been associated with the development of primary congenital glaucoma, an autosomal recessive disease 10, 11), indicating that CYP1B1 has a physiological function in addition to PAH bioactivation. Two frequently occurring polymorphisms result in amino acid changes in codon 432 (Val → Leu) and codon 453 (Asn → Ser) (12). Based on the extensive DNA sequencing analysis of the *CYP1B1* gene in over 100 individuals in the published reports, it seems unlikely that there are additional polymorphisms in the *CYP1B1* coding region. Therefore we modified the original task slightly and analysed our specimens for the known *CYP1B1* polymorphisms.
The 432 Val → Leu and 453 Asn → Ser genotypes were determined in all specimens according to the procedure described by Bailey et al. (12).

Table II *CYP1B1* genotypes of cases and controls

CYP1B1 genotypes*	Cases	Controls
wt/wt	6	10
m1/wt	13	10
m1/m2	7	12

* m1 is 432 Val → Leu, m2 is 453 Asn → Ser. Heterozygote and homozygote genotypes were combined, because of the few samples

Because of the very low number of samples analysed so far, it is impossible to determine if the variant genotypes occur at different frequency in cases and controls. But we conclude from our study that the 453 Ser variant genotype is strictly linked to the 432 Leu variant genotype, a so far unreported observation. The *CYP1B1* genetic variants studied here have been shown to have an increased estrogen hydroxylation activity compared to the wildtype genotypes (13, 14). Given the carcinogenic and estrogenic potential of 4-hydroxy estradiol, the inheritance of variant*CYP1B1* genotypes could contribute to interindividual differences in breast cancer risk. A recent study found that the 432 Leu genotype is in fact associated with increased risk of breast cancer (15).

CYP1A1: Four polymorphisms consisting of single base changes have been identified in the *CYP1A1* gene (9) and two of the polymorphic variants (MspI and Ile-Val) have been associated with increased risk of lung cancer in Japanese, though it is unclear whether these polymorphisms affect the inducibility and activity of CYP1A1 (16).
To investigate to what extent the *CYP1A1* genotype modifies *CYP1A1* expression, the *CYP1A1* genotype of all specimens was determined using PCR/RFLP analysis as described in the attached manuscript. A total of 8 MspI and Ile-Val variants were detected in the 58 samples, 7 in the cancer free group and one in the patient group. When all *CYP1A1* values were ranked, the *CYP1A1* variants were distributed between the lowest and highest expression values with all heterozygote variants and one of the two homozygote variant having *CYP1A1* values below the mean *CYP1A1* values. These findings indicate that the *CYP1A1* polymorphism has at most a minor role in determining the *CYP1A1* expression level.

Future goals
Our study groups were quite small. We plan to expand the sample size of both groups in the coming year. Recently we were very fortunate in that we were offered an extensive tissue bank maintained by Aeron Biotechnology Inc. This tissue bank had been established by Peralta Cancer Center in 1981-1988 and contains breast tissue specimen from 180 individuals, including tissue from 80 mastectomies, 50 tumor excisions and 40 reduction mammoplasties. We transfered all specimens stored in liquid nitrogen from to the laboratory. This bank will be a valuable resource to further test our hypothesis that certain genotype variants or overexpression of genes represent risk factors for breast cancer.

Key Research Accomplishments

- *CYP1B1* and*CYP1A1* expression was measured in an additional 30 nontumor breast tissue specimens. The results confirmed the conclusions from analysis of the first 30 specimens i.e. (a) that in most specimens *CYP1B1* is expressed at considerably higher levels than *CYP1A1* , indicating that the CYP1B1 enzyme is primarily responsible for PAH activation in breast tissue; and (b) that *CYP1B1* expression is significantly higher in the breast cancer patients than in healthy individuals.

- All available specimens were analysed for two known*CYP1B1* genetic polymorphisms. The sample size was too small to make prediction about the association of variant genotypes with disease. We did find that the 453 Asn → Ser variant genotype is strictly linked to the 432 Val → Leu variant genotype.

- The specimens were analysed for two *CYP1A1* genetic polymorphisms. The *CYP1A1* variant genotype was not associated with high *CYP1A1* expression.

Reportable Outcomes

Peer reviewed publication:

R. Goth-Goldstein, C.A. Erdmann, M.R. Stampfer, M.L. Russell. Interindividual Variation in CYP1A1 Expression in Breast Tissue and the Role of Genetic Polymorphism, Carcinogenesis, in press.

Abstracts and presentations:

1. Invited talk on 'Metabolism of Environmental Chemicals as Breast Cancer Risk' at the California Breast Cancer Research Symposium, 9/18/1999 in Los Angeles.

2. Poster presentation at AACR 4/00, San Francisco by R. Goth-Goldstein, C. Erdmann, M. Russell 'CYP1B1 expression in normal human breast tissue specimen' Proc. Am. Ass. Cancer Res.41 # 807 (2000).

3. Poster presentation at a meeting on 'New Frontiers in Women's Health Research' at the UC Davis Cancer Center, 4/00 entitled 'Variation in metabolism of carcinogens as a risk factor for breast cancer'.

4. Poster presentation 'Interindividual Variation in Metabolism of Carcinogens as a Risk Factor for Breast Cancer' at the DoD-sponsored meeting 'Era of Hope' in Atlanta in June 2000

Conclusions

Because of the potential important role of CYP1B1 in the activation of environmental and endogenous compounds to carcinogenic intermediates, it was hypothesized that high CYP1B1 expression could represent a risk factor for breast cancer. This is the first study to measure expression of *CYP1B1* in a collection of nontumor breast tissue from mastectomy patients and from reduction mammoplasties, to estimate the interindividual variation of CYP1B1 levels and to compare expression in breast cancer patients and healthy individuals. We found a large interindividual variation in *CYP1B1* expression. CYP1B1 transcript levels were 2-7 fold higher than CYP1A1 in most samples indicating that CYP1B1 is the predominant PAH-metabolizing enzyme in the breast. *CYP1B1* expression was higher in the breast cancer group compared to the control group and the difference was statistically highly significant. *CYP1A1* and *CYP1B1* genotype variants were determined, but there was no conclusive difference between cases and controls.

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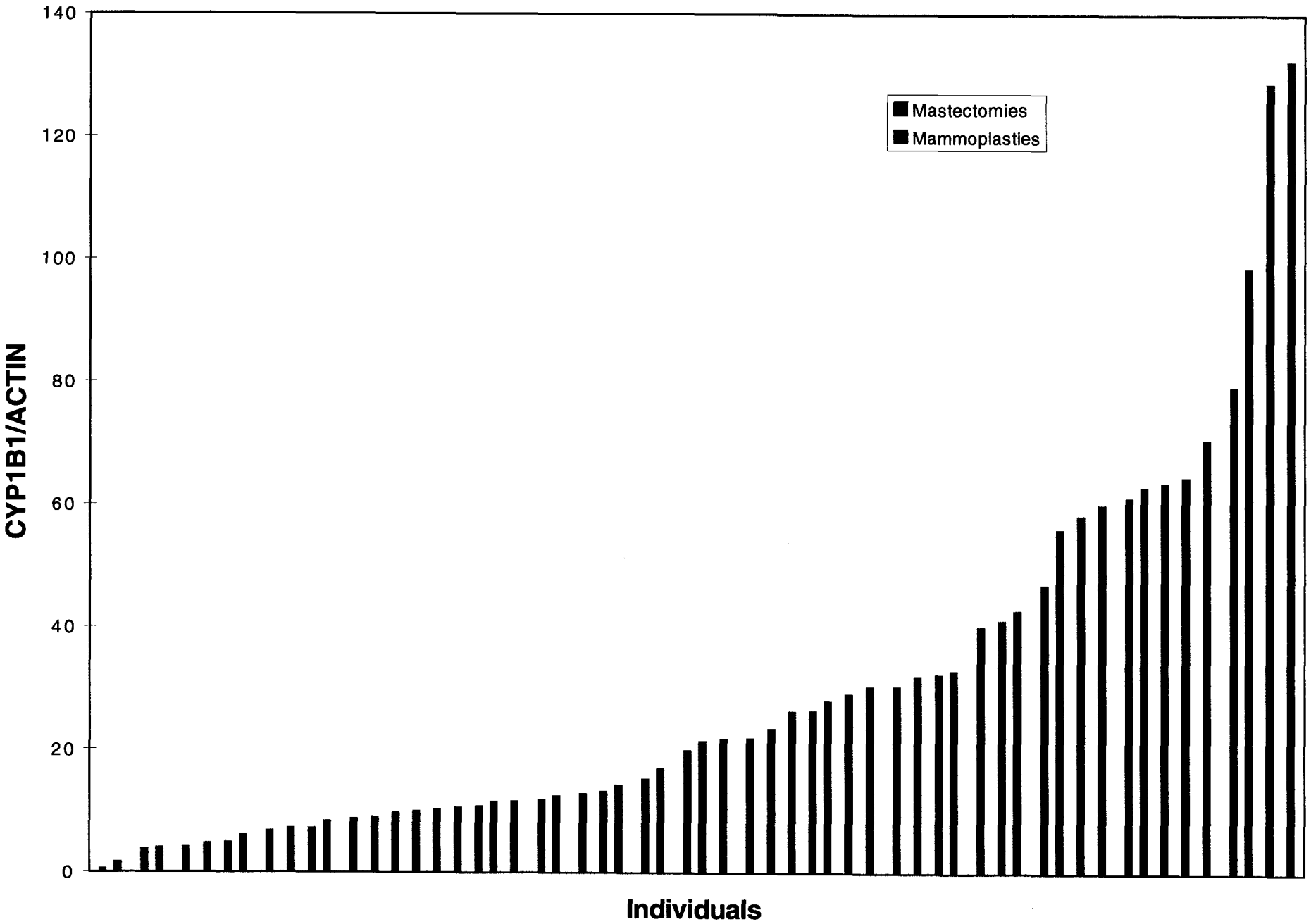
APPENDIX

1. Figure 1: *CYP1B1* expression (relative to ß-actin) in non-tumor breast tissue of cases and controls ranked by increasing values.

2. Figure 2: Comparison of *CYP1B1* and *CYP1A1* expression in each specimen.

3. Figure 3: Distribution of *CY1B1* expression values in cases (top panel) and controls (bottom panel).

4. Manuscript to appear in *Carcinogenesis.*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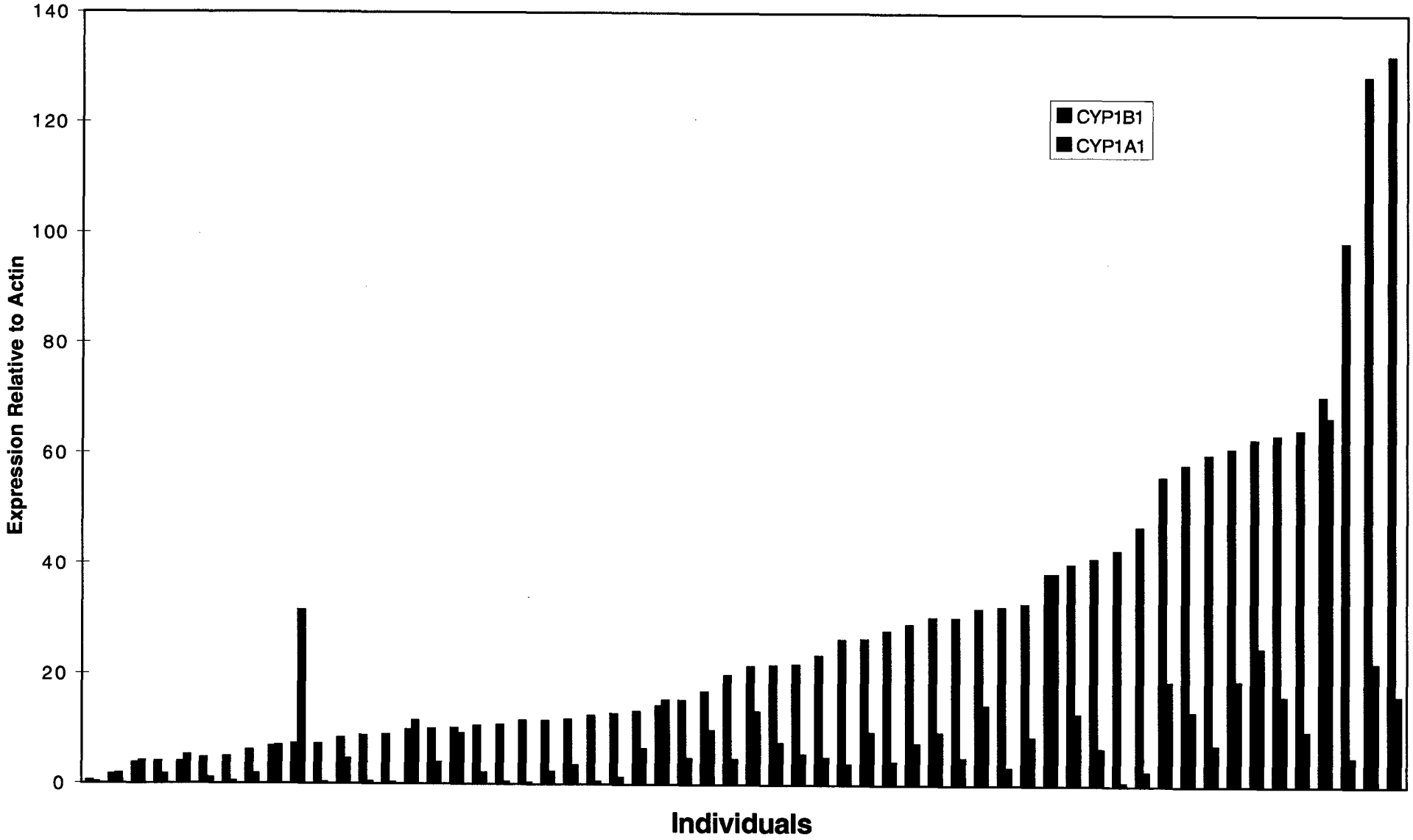
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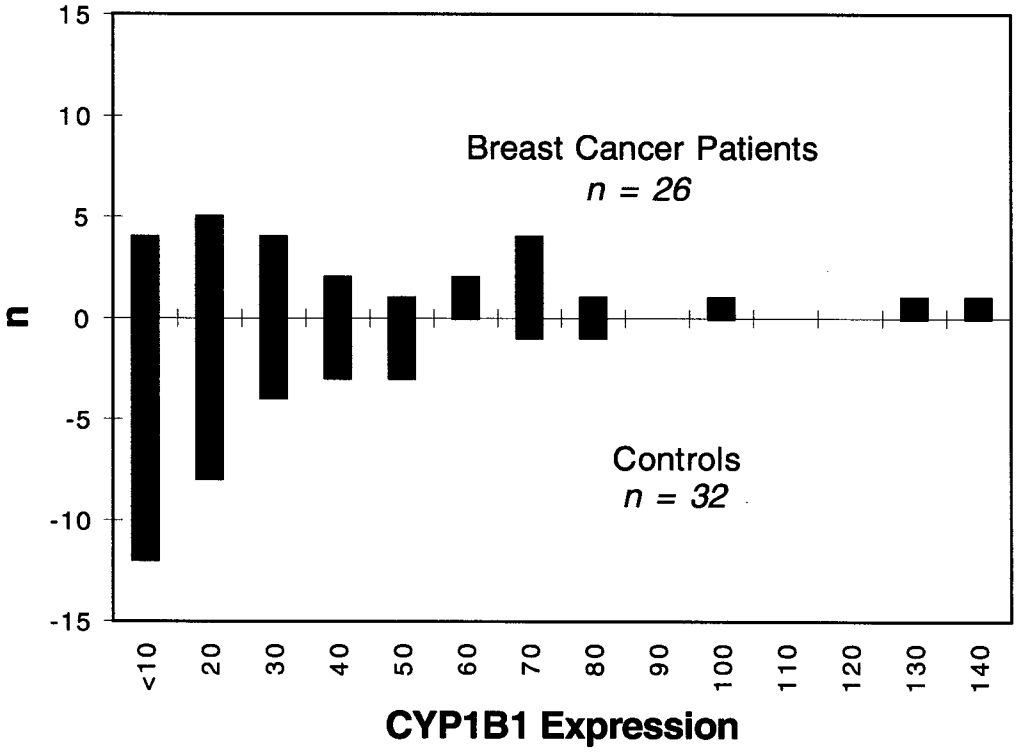
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CYP1B1 Expression

Interindividual Variation in *CYP1A1* Expression in Breast Tissue and the Role of Genetic Polymorphism

Regine Goth-Goldstein[1,4], Martha R. Stampfer[2], Christine A. Erdmann[3] and Marion Russell[1]

Lawrence Berkeley National Laboratory, [1]Environmental Energy Technology Division, [2]Life Sciences Division, [3]Information and Computing Sciences Division, One Cyclotron Road, Berkeley, CA 94720

[4] To whom correspondence should be addressed at Lawrence Berkeley National Laboratory, Mail Stop 70-108B, One Cyclotron Road, Berkeley, CA 94720, Phone: (510) 486-5897; Fax: (510) 486-7303; E-mail: R_Goth-Goldstein@lbl.gov

ABSTRACT

The cytochrome P4501A1 (CYP1A1) enzyme is regulated at the transcriptional level and its expression is influenced by genetic factors, polymorphisms in the structural and regulatory genes, and by environmental factors such as exposure to polycyclic aromatic hydrocarbons (PAHs). To investigate the role of CYP1A1 in breast cancer, we studied *CYP1A1* in breast tissue at the level of expression, thereby taking all possible modifying factors into account. We measured *CYP1A1* expression in 58 non-tumor breast tissue specimens from both breast cancer patients (n = 29) and cancer-free individuals (n = 32) using a newly developed RT-PCR assay. *CYP1A1* expression varied between specimens about 400-fold and was independent of age. *CYP1A1* expression was somewhat higher in tissue from breast cancer patients than cancer-free individuals, but this difference was not statistically significant. Analysis for *CYP1A1* genetic polymorphisms revealed 8 variants, 7 in the cancer free group and one in the patient group. The variant genotype did not predict well the expression level. We conclude that high CYP1A1 expression is possibly a risk factor for breast cancer and that the known *CYP1A1* polymorphisms are not good predictors of *CYP1A1* expression.

Abbreviations

Ah receptor, aryl hydrocarbon receptor; BaP, benzo(a)pyrene; CYP1A1, cytochrome P4501A1; HMEC, human mammary epithelial cells; PAHs, polycyclic aromatic hydrocarbons; RT-PCR, reverse transcription PCR.

Running Title: CYP1A1 Expression in Breast Tissue

Polycyclic aromatic hydrocarbons (PAHs), a class of chemicals which includes potent carcinogens, could have a role in breast cancer because they accumulate in breast adipose tissue (1), and because normal human mammary cells in culture activate PAHs efficiently (2). PAH-DNA adduct levels have been found to be significantly higher in normal breast tissue of breast cancer patients compared to non-cancer controls (3). The mutational spectrum in the p53 gene in breast tumors resembles that of lung cancers where there is a well-established role for environmental agents, such as tobacco smoke (4). The major metabolic pathway for ingested or inhaled PAHs to water-soluble derivatives is oxidative activation by CYP1A1 followed by detoxification by phase II enzymes. There is evidence supporting a role of CYP1A1 in breast cancer from recent animal experiments: using a rat model to identify loci that control breast cancer susceptibility, one of the four loci mapped to *CYP1A1* or a nearby locus (5).

Interindividual variation in carcinogen metabolism has been recognized as a determinant of susceptibility to various cancers (6). Genetic polymorphism is one potential source of variation. For *CYP1A1* four genetic polymorphisms consisting of single base changes have been described (7), two of them have been studied extensively as genetic biomarkers of susceptibility to various cancers (6) including breast cancer (8). The first described variant (*CYP1A1*2*) is located in the 3'noncoding region of the *CYP1A1* gene and introduces an *Msp*I endonuclease site (9, 10). The second variant (*CYP1A1*3*) is strictly linked to *CYP1A1*2* (7) and consists of an A to G transition in exon 7 that results in an amino acid substitution of Val^{462} to Ile^{462} (11). Even though several studies suggested that this genotype increases susceptibility to various cancers, the biochemical basis is unclear. It has been assumed that the *CYP1A1*2* and *CYP1A1**3 alleles lead to higher inducibility. Expression of *CYP1A1* is regulated by the Ah receptor, together with several other regulatory proteins. Increased transcription of the *CYP1A1* gene reflects induction of the enzyme (12). *CYP1A1* expression can be induced by exposure to PAHs and organochlorines (13). Besides environmental factors, genetic factors

can modify *CYP1A1* expression such as the genotype of the structural gene and the genotype of regulatory genes, including the Ah receptor. Therefore determining the transcript or the actual level of the enzyme, captures the influence of all potentially modifying factors and represents a more sensitive tool than the genotype of a single gene.

We have examined *CYP1A1* expression as a possible breast cancer risk factor by comparing CYP1A1 expression in non-tumor breast tissue from 27 breast cancer cases and 32 cancer-free individuals. Although we did not measure CYP1A1 protein levels or CYP1A1 enzyme activity, mRNA levels and enzyme activities are known to be closely related (14, 15). The case specimens were derived from 22 mastectomies (peripheral nontumor tissue) and 5 contralateral to carcinomatous breast. The control specimens were obtained from 32 reduction mammoplasties. Tissue specimen were dissected and isolated from adipose and connective tissue, so that only epithelial material was stored frozen as organoids (16). The pathological diagnosis of the excised tumors was intraductal carcinomas for 2 cases and infiltrating ductal carcinoma for the other 20 cases. In two of the 22 cases, metastasis to axillary lymph nodes were observed indicating more advanced disease. Samples were collected without respect to age and race. Only the age and disease status of the specimen donors are known. No information is available on donors' race, lifestyle, smoking habits or other potential confounding factors. Individuals undergoing reduction mammoplasty ranged in age from 15 to 68 years, and mastectomy patients ranged from 30 to 87 years.

To quantitate CYP1A1 transcript levels, we developed an RT-PCR assay that determines *CYP1A1* expression relative to the constantly expressed ß-actin gene, thus controlling for varying sample sizes and RNA yield. Previously published primers designed to span an intron (thus excluding amplification of any contaminating genomic DNA) were used and generated products of 320 base pairs for CYP1A1 and 273 base pairs for ß-actin (17, 18). PCR conditions and cycle numbers were optimized separately for each target sequence to

ensure that the reaction is in the linear phase of product accumulation. A five-fold serial dilution of cDNA was amplified in separate reactions for CYP1A1 and ß-actin. After amplification, the products were mixed together before electrophoresis on a 10% native polyacrylamide gel. The gel was stained with SYBR Gold nucleic acid stain and scanned on a Molecular Dynamics STORM 860 optical scanner. The fluorescent signal for each band was quantitated using ImageQuant software (Figure 1). We found that this assay for *CYP1A1* expression is sensitive, reproducible and has a broad dynamic range. *CYP1A1* expression was measured in 59 non-tumor breast tissues from individuals with breast cancer (n = 27) and from cancer-free individuals (n = 32). Only 1 out of the 59 samples did not have amplifiable RNA. CYP1A1 quantitation was repeated in a blinded assay for 20 % of samples. The correlation between the original measurements and the respective repeats was 0.9878 indicating that the assay is highly reproducible. In experiments with human mammary epithelial cells in culture, we found that the ß-actin transcript level is independent of BaP exposure, whereas CYP1A1 transcript levels increase in a dose-response fashion (data not shown). In the present study, ß-actin transcript levels in the 58 specimens could be evaluated from one of the first two dilutions of the cDNA. In contrast, the whole range of dilutions was needed to determine the CYP1A1 transcript levels in all specimens, indicating the large interindividual variations in *CYP1A1* expression. The *CYP1A1* to ß-actin ratio varied between the lowest value of 0.17 to the highest value of 70, a more than 400-fold range. As seen in Figure 2, individuals in the control group were younger than in the case group, but *CYP1A1* expression did not change with the age of the donors. The correlation coefficient for CYP1A1 to ß-actin ratio and age is -0.0357 for cancer patients and 0.0434 for controls, constituting persuasive evidence that CYP1A1 level and age are not correlated. The lack of a correlation with age indicates that the reduction in estrogen levels experienced with menopause does not influence the CYP1A1 level, even though an interaction between the Ah receptor and the estrogen receptor pathways has been observed in several systems (13)

CYP1A1 expression represented by the *CYP1A1* to ß-actin ratio differed between groups: The arithmetic mean of the CYP1A1/ß-actin ratio was 9.55 (SD = 14.66) in specimens from breast cancer patients and 6.31 (SD = 6.91) in specimens from cancer-free individuals. This difference was not statistically significant (in two-tailed t-test t = -1.11, p = 0.27) in the small sample studied. Comparing the distribution of CYP1A1/ß-actin values, a fairly log-normal distribution of values is seen for cases and controls (Figure 3). The geometric mean of the CYP1A1/ß-actin ratio was 3.70 (SD = 4.90) in cases and 3.15 (SD = 4.05) in controls.

The large interindividual variation of *CYP1A1* expression might be explained by unmeasured environmental or lifestyle factors, such as smoking, which is known to induce *CYP1A1* expression. *CYP1A1* expression is increased in lung tissue of patients with tobacco-induced lung cancer (19). Others have reported variation in *CYP1A1* expression in lung tissue (15, 20, 21), including a recent report that found a more than two-fold higher *CYP1A1* expression in females than males (22).

The *CYP1A1*2* and *CYP1A1*3* alleles have been associated with highly inducible phenotype in vitro (11). To investigate to what extent the *CYP1A1* genotype modifies *CYP1A1* expression, the *CYP1A1* genotype of all specimens was determined using PCR/RFLP analysis according to published procedures (7). A total of 8 *CYP1A1*2* and *CYP1A1*3* alleles in 58 samples were detected, 3 *CYP1A1*2* heterozygotes, 3 *CYP1A1*2/ CYP1A1*1* heterozygotes and 2 *CYP1A1*2* homozygotes. The patient group had only one *CYP1A1*2/ CYP1A1*1* heterozygote while the control group had 7 variants. When all *CYP1A1* values are ranked (Figure 4), the *CYP1A1* variants are distributed between the lowest and highest expression values. All heterozygote variants and the one homozygote *CYP1A1*2*

variant have *CYP1A1* values below the mean *CYP1A1* values. Only one homozygote *CYP1A1*2* variants is among the 5 specimen with the highest *CYP1A1* expression values, indicating that the polymorphism has at most a minor role in determining the *CYP1A1* expression.

The *CYP1A1*2* variant is located in the noncoding region of the gene, suggesting that the *CYP1A1*2* polymorphism alters the inducibility of *CYP1A1*. The *CYP1A1*3* variant is located in exon 7 which codes for the heme binding region. A change in amino acids in this region could possibly result in a change in enzyme activity. An earlier study reported a 50% higher enzyme activity (11). However, using purified human recombinant CYP1A1*1 and CYP1A1*2, a more recent study did not find different BaP activation (23). Another study reported no difference in the kinetics of the CYP1A1 polymorphic variants (24). Therefore, any change in CYP1A1 level in *CYP1A1*3* seems to be the result of strict linkage to *CYP1A1*2* polymorphism (7), which presumably alters the inducibility of the enzyme. Our data suggest that *CYP1A1*2* polymorphism has a minor, if any role in modifying *CYP1A1* expression (Figure 4). If individuals with the *CYP1A1* variant genotype were exposed to much lower levels of PAHs than individuals with the wild type genotype, the impact of genotype on expression might be masked. In an earlier study, human mammary epithelial cells derived from 18 individuals were treated with benzo(a)pyrene and DNA adducts quantified (2). Among the strains examined were six derived from donors tested here for *CYP1A1* expression and *CYP1A1* genotype, including the two homozygous *CYP1A1*2* and one of the heterozygous *CYP1A1*2* variant identified here. Contrary to expectations, the two homozygous *CYP1A1*2* alleles had the lowest amount of adducts, indicating that the *CYP1A1*2* genotype did not increase DNA adduct formation. Besides activating xenobiotics, CYP1A1 also metabolizes 17 β-estradiol to the less active 2-hydroxy estradiol (25). A recent

study suggests that *CYP1A1*2* may be a marker of altered estradiol metabolism and of increased susceptibility to estrogen-related breast cancer in African-Americans (26).

In conclusion, this study shows that breast tissue expresses a considerable range of *CYP1A1* levels independent of age and genotype, reinforcing the importance of evaluating both genotype and phenotype. Although the results are not statistically significant in the small unselected specimen groups available, they suggest that increased PAH activation by *CYP1A1* might play a role in initiation of breast cancer. Larger sample sizes will be required to corroborate these suggestive findings.

Acknowledgments

We thank Dr. Paul Williams for help and advice with the statistical analysis. We also thank Dr. Leeka Kheifits of EPRI for critical review of the manuscript. This research was supported by funds from the California Breast Cancer Research Program of the University of California, Grant Number 1RB-0429 (RGG), by USAMRMC Grant Number DAMD17-98-1-8062 (RGG) and by the National Cancer Institute, Grant Number CA-24844 (MRS). The work was performed under US Department of Energy Contract No. DE-AC03-76SF00098.

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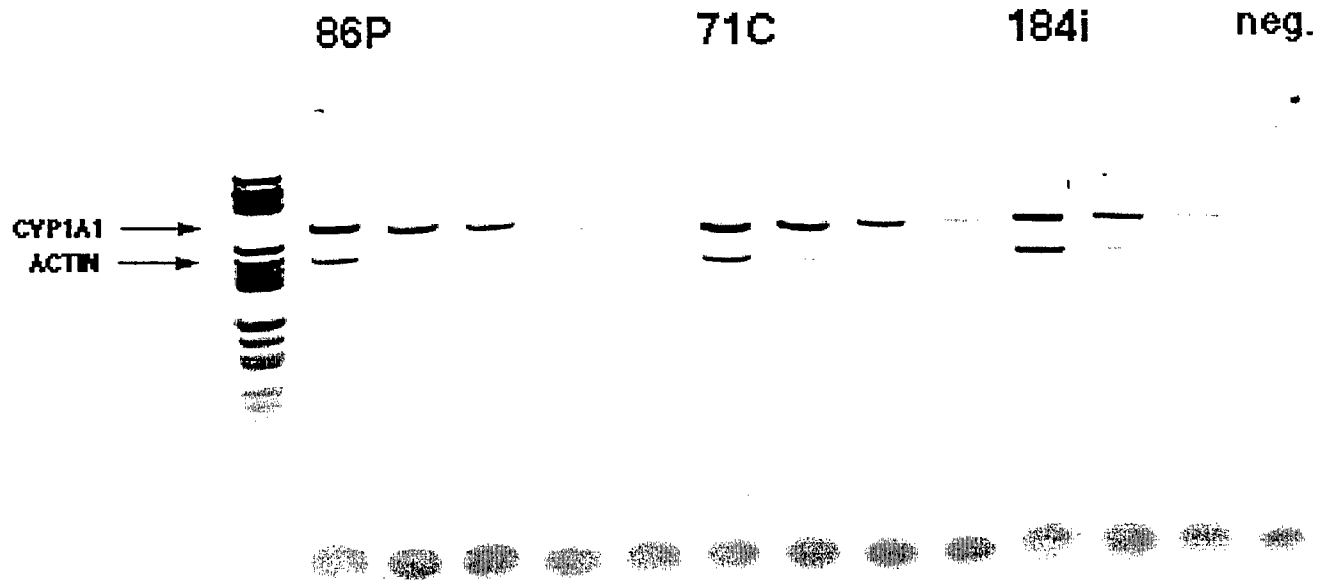
Figure Legend

Fig. 1: Polyacrylamide gel of quantitated CYP1A1 and β-actin PCR products for 3 specimens. The cDNA from each specimen was diluted serially 5-fold and several of these dilutions were amplified for each specimen. Lane 1, molecular weight standard; lane 2-6 specimen 86P peripheral to tumor; lane 7-10, specimen 71C contralateral; lane 11-13, 184 cells included in each reaction as control to test for interexperimental variation; lane 14, negative control.

Fig. 2: CYP1A1 to ß-actin ratio as function of age of specimen donors; □ represent values of reduction mammoplasty controls, ● represent values of breast cancer cases.

Fig. 3: Distribution of CYP1A1 expression levels among breast cancer cases and reduction mammoplasty controls. The upper two histograms show the untransformed data. The bottom two histograms show the log-transformed data.

Fig. 4: CYP1A1 to ß-actin ratio ranked for all individuals. Open bars represent the *CYP1A1 *1* (wild type) genotype. Solid bars represent *CYP1A1* polymorphic variants of the following categories: a. *CYP1A1*2* heterozygotes; b. *CYP1A1*2/CYP1A1*1* heterozygotes; c. *CYP1A1*2* homozygotes. CYP1A1 to ß-actin ratios are given in parentheses for the polymorphic variants. The origin of each tissue specimen is given below the bar, R - reduction mammoplasty, P - peripheral to carcinoma, C - contralateral.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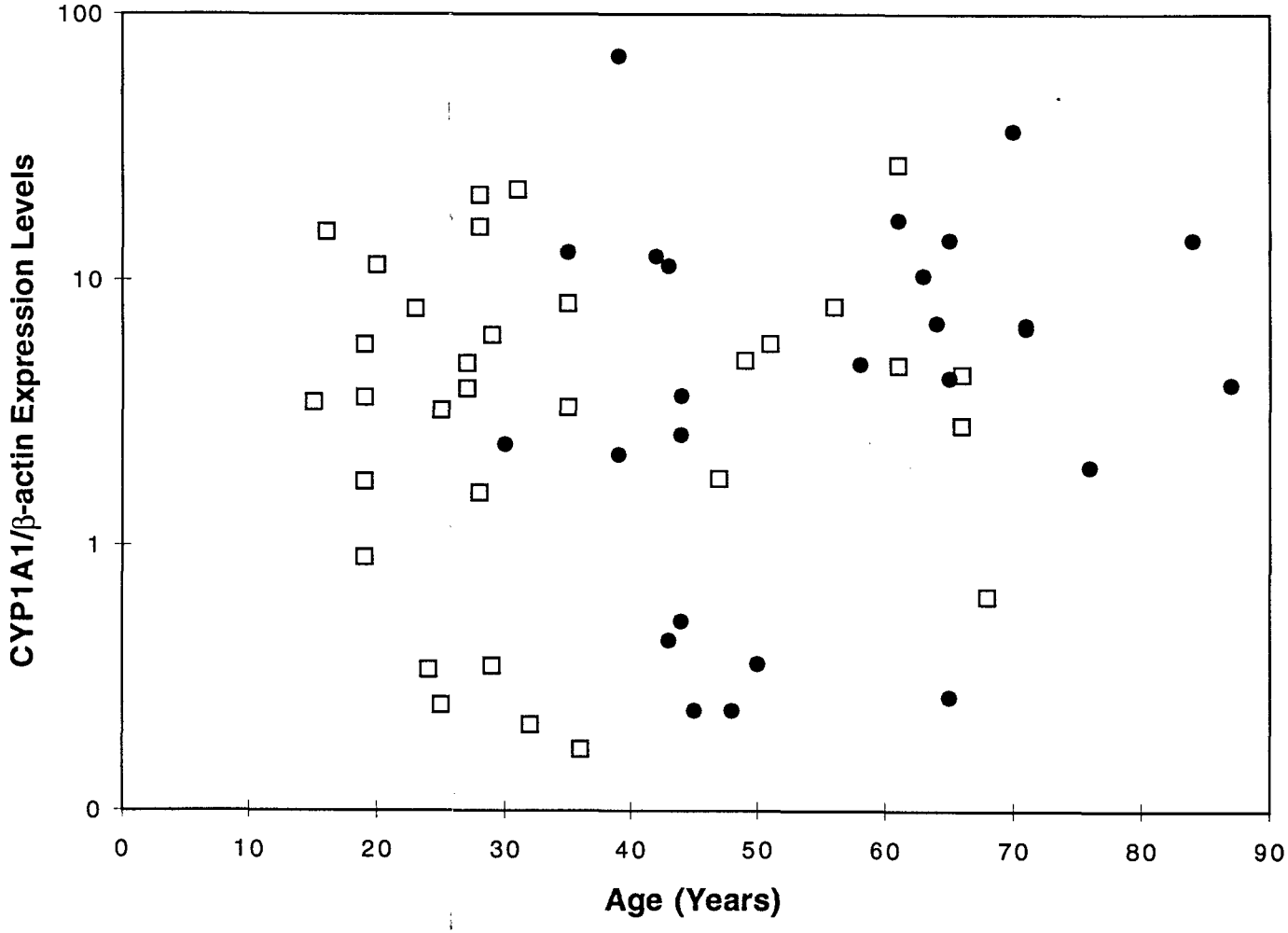
100
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80
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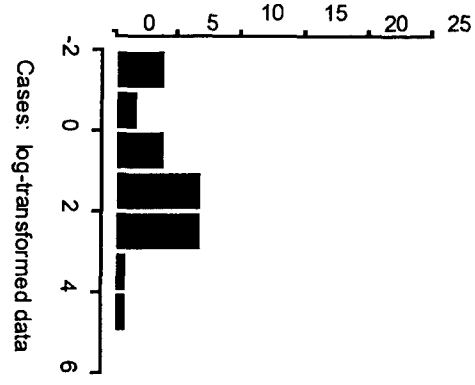
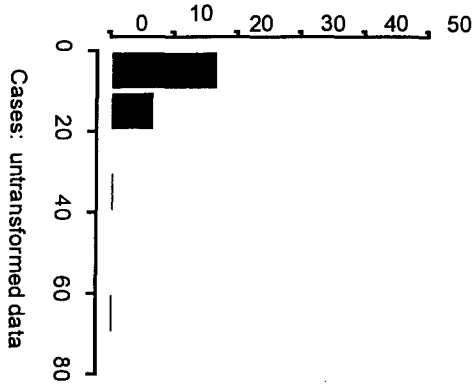
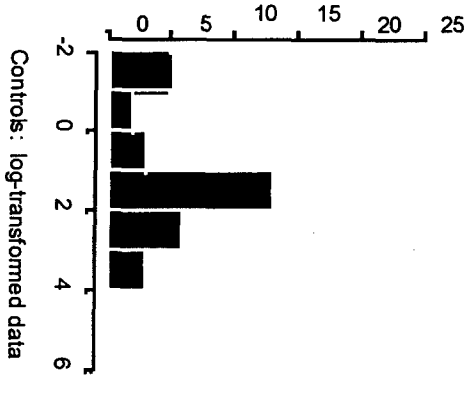
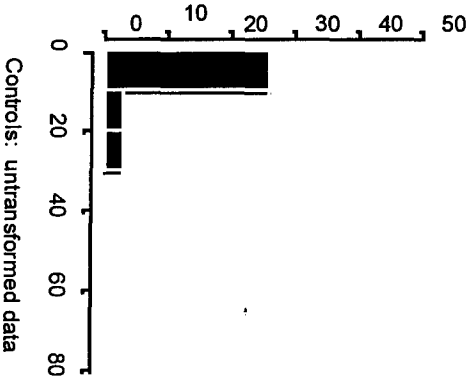
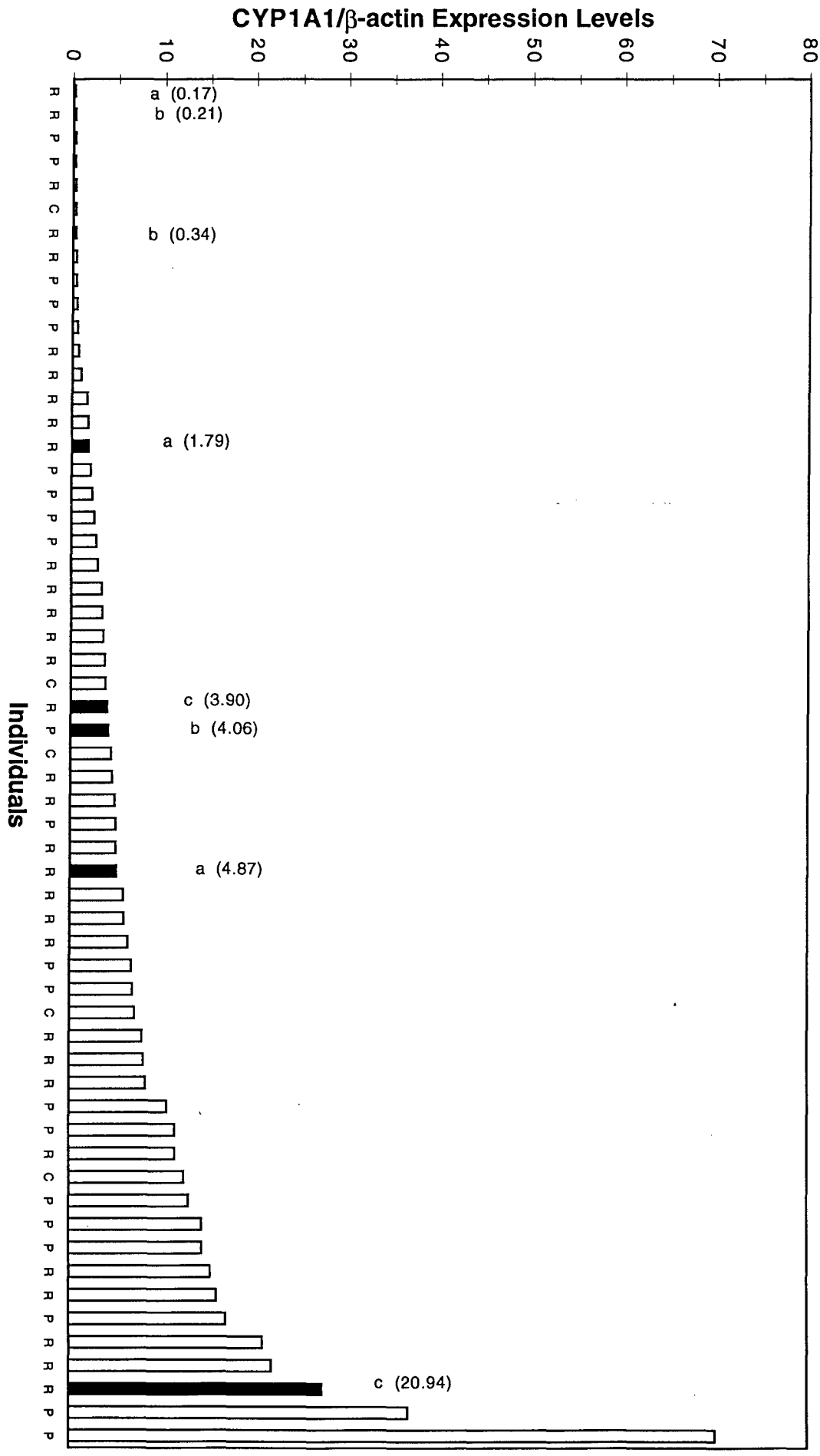
Age (Years)


0 5 10 15 20 25
-2 0 2 4 6
Cases: log-transformed data


0 10 20 30 40 50
0 20 40 60 80
Cases: untransformed data


0 5 10 15 20 25
-2 0 2 4 6
Controls: log-transformed data


0 10 20 30 40 50
0 20 40 60 80
Controls: untransformed data


CYP1A1/β-actin Expression Levels
0
10
20
30
40
50
60
70
80
Individuals
a (0.17)
b (0.21)
b (0.34)
a (1.79)
c (3.90)
b (4.06)
a (4.87)
c (20.94)

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